

Serial No.: 09/020,716
Group Art Unit:

Support for elevated level of preselected amino acid is from expression of modified barley alpha hordothionin, 2S albumin proteins and derivatives of each protein in the endosperm is found on page 9, line 6- page 13, line 8.

Claims 2 and 4 have been cancelled.

Objections

Claims 1 and 5 are objected to by the Examiner because of informalities.

In Claim 1, the Examiner suggests replacing "is characterized as having" by --has--.

Applicant respectfully reminds the Examiner that the M.P.E.P. 2111.03 states that the phase "characterized by" is synonymous with "including," "containing," and is inclusive or open-ended.

In Claim 5, the Examiner suggests that "oats" should be changed to --oat--.

Applicant has rewritten original claim 5, as new claim 23 to reflect the Examiner's suggested change.

Rejection Under 35 U.S.C. §112, first paragraph

Claims 1-21 are rejected by the Examiner under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The Examiner states that the claimed invention is drawn broadly toward a transformed cereal plant seed with an elevated level of at least one amino acid and that the Applicant does not describe other transformed cereal plant seed modified in other amino acids transformed with other modified or unmodified genes, and hence it is not clear from the instant specification that the Applicant was in possession of the invention as broadly claimed.

The instant application broadly describes transformed cereal plants with elevated levels of the preselected amino acids lysine and a sulfur containing amino acid.

The instant specification describes the rational substitution of methionine for surface hydrophobic amino acid residues such as leucine and valine and for surface polar amino acids asparagine, glutamine and threonine (page 11, lines 9-16). Also described is

Serial No.: 09/020,716
Group Art Unit:

methionine substitutions for charged amino acids (page 11, lines 19-20)

Description of the modifications of hordothionin is found from the top of page 9 through the bottom of page 11 of the instant specification. The modifications are also found in Seq. ID Nos 3-5.

Support for soybean 2S albumin (ESA) is found on page 12, line 1-5 with elevated levels of preselected amino acids. Methods for modifying ESA are incorporated by reference.

The Examiner suggests looking at University of California V Eli Lilly and Co., 43 U.S.PQ2d 1398 (Fed. Cir. 1997), which teaches that the disclosure of a process for obtaining cDNA from a particular organism and the description of the encoded protein fail to provide an adequate written description of the actual cDNA from that organism which would encode the protein from that organism, despite the disclosure of a cDNA encoding that protein from another organism.

Applicant respectfully points out that specific DNA sequences are not being claimed. The instant application claims any DNA encoding a protein with elevated levels of preselected amino acids p. 6-13.

Applicant submits that the rationale for changing amino acid residues is detailed on pages 9-11 and examples are provided describing modifications.

Further support for the DNA sequences of HT12, and ESA are found on page 30, lines 1-19 and page 40 (Table 2) as PHP7999 (Seq. ID No. 4) and PHP5025 (Seq. ID No. 5), and PHP11260 (Seq. ID 6).

Rejection Under 35 U.S.C. §112, first paragraph

The Examiner states that claims 1-21 are rejected under 35 U.S.C. 112, first paragraph, because the specification is enabling only for claims limited to transformed cereal plant seed having an elevated lysine content comprising the modified hordothionin gene of SEQ ID NO:2 (HT12), vectors, plant cells and transformed plants comprising said modified hordothionin gene, and a method for increasing seed lysine content by transformation with said modified hordothionin gene. He goes on to say that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with

Serial No.: 08/020,716
Group Art Unit:

these claims.

Applicant submits that the teachings in the specification can be used to increase levels of lysine and a sulfur containing amino acid in cereal plants. Steps for modeling, modification and testing Hordothionin are outlined on pages 9-11 and in the examples and can be extrapolated to other polypeptides.

The MPEP states "compliance with the enablement requirement of 35 U.S.C. 112, first paragraph does not turn on whether an example is disclosed....An applicant need not have actually reduced the invention to practice prior to filing. Gould v. Zuigg, 822F.2d 1074, 1078, 3 U.S.PQ 2d 1302, 1304 (Fed. Cir. 1987)."

The supporting declaration by Rudolf Jung describes Hordothionin and ESA (enhance soybean albumen derived polypeptides) with elevated levels of preselected amino acids lysine and a sulfur containing amino acid.

The Examiner states that the state of the art for amino acid substitution is highly unpredictable. In particular, the role of specific amino acids in protein function cannot be reliably predicted, and the effect of amino acid substitution on protein activity must be determined empirically.

It is submitted that directions for specific amino acid substitutions in the modified hordothionin and soybean 2S albumin (ESA), are provided in the instant application, as described *supra* or incorporated by reference therein. Rational substitution, as detailed in the instant specification, provides a method of extrapolating and making reasonable changes in a polypeptide to effect changes in amino acid composition.

The Examiner states that the state of the art for modification of gene expression or of phenotypic characteristics in plants by genetic transformation is highly unpredictable and hence significant guidance is required to practice the art without undue experimentation. The specific effects of given promoters, leaders, DNA sequences, and terminator sequences on gene expression in transformed plants can not be anticipated reliably and must be determined empirically (Plant Mol. Biol. 32: 393-405, 1996, abstract, pp.402-403).

It is submitted that neither full function nor full activity are stated as being necessary for elevated levels of preselected amino acids in the instant application.

The accompanying declaration of Rudolf Jung provides evidence of both

Serial No.: 09/020,716
Group Art Unit:

hordothionin and ESA expression using the gamma zein promoter.

Specific examples of "given promoters, leader, DNA sequences and terminator sequences" are provided in the instant application. Preferred promoters are seed endosperm-preferred promoters (page 17, lines 20-23 and page 18, lines 7-18). The gamma zein terminator (page 29, lines 16-17), glob 1 terminator (page 30 lines 1-3) and waxy terminator (page 30, line 4-5) are described using hordothion as an example. ESA is also described with either glob, waxy, gamma zein promoters and terminators (page 30, lines 9-19 and page 40, Table 2). The sequences are referenced, *supra*.

The Examiner states that in genetically modified plants, the introduced transgenes are sometimes not expressed, and they can also result in co-suppression effects. None of these effects are predictable, and the mechanisms of gene silencing are still not fully understood (Ann. Bot. 79: 3-12, 1997, abstract, p.9). Moreover, the phenotypic characteristics that will result from expression of a given DNA construct can not be reliably predicted. In fact, often the expected phenotypic result is not achieved. For example, antisense expression of polygalacturonase gene in transgenic tomato had no effect on fruit softening (Nature 334: 724-726, 1988, p.725).

Applicant submits that modified hordothionin and ESA transgenes were expressed and levels of preselected amino acids lysine and a sulfur containing amino acid were elevated as shown in the accompanying declaration of Rudolf Jung.

Selection of the desired phenotype is required with any transformation event and methods are known in the art and described in the instant specification for so doing.

The Examiner states that given the unpredictability in the art, the instant invention is not enabled given the lack of guidance in the specification with regard to what amino acid substitutions other than the 12 amino acid substitutions of SEQ ID NO:2 in what gene other than the barley hordothionin gene can be expressed in a transgenic plant, resulting in an elevated level of an amino acid other than lysine. Applicant provides no guidance for other locations for and types of amino acid substitutions in the barley hordothionin gene which would not result in a loss of protein activity and which would result in an increase in the level of the substituted amino acid upon transformation of plants. In the absence of such guidance, undue trial and error experimentation would be required to screen through the

Serial No.: 09/020,716
Group Art Unit:

vast number of potential amino acid substitutions to determine the locations and amino acids which could be successfully substituted.

Applicant submits that neither full function nor full activity are stated as being necessary for elevated levels of preselected amino acids in the instant application as discussed *supra*.

The Examiner states that although Applicant briefly mentions the *ESA* and the *BHL* genes, Applicant does not provide specific guidance with respect to locations of and amino acids which could be substituted, resulting in an enhanced level of the substituted amino acid in transgenic plants.

Applicant submits that specific guidance with respect to locations of and amino acids which could be substituted is found in the instant specification on page 9-11 and in US Patent No. 5,850,016, issued Dec. 15, 1998, and in Coulter et al, both which are incorporated by reference. Information is also provided in patent cited and incorporated by reference in the instant specification.

Also, the Examiner states that although Claims 10-12 are indefinite as discussed below, it does not appear that Applicant has provided guidance for the specific elevated levels of lysine. Whereas Applicant teaches some increase in lysine content maize kernels when the mutant *HT12* gene is expressed behind the waxy or zein promoter in transgenic maize plants (Table 1), it is not clear that the increases are greater than 20% by weight, for example, especially because the units of measurement in are not clear from the table.

Table 1 results are described in the instant specification on page 39, lines 8-17 as "...amino acid composition of whole kernels..." The percent of amino acid that is lysine increase from 0.29% in wild-type to 0.38% or 0.39% for the waxy and zein constructs, respectively. This represents an increase of 31-34.4%. It is standard in the art is to measure the total dry weight of the meal.

The accompanying declaration shows samples of the *HT12* transgenics (with the gamma zein promoter) with at least a 10% increase, or at lease a 15% increase or at least a 20% increase in lysine and in a sulfur containing amino acids over the controls. Four out of 14

Serial No.: 09/020,716
Group Art Unit:

of the transgenics show at least a 20% increase in lysine and in a sulfur containing amino acids over the control.

The Examiner states that given the unpredictability in the art, the scope of the claimed invention is not commensurate with the teachings of the specification, and hence the invention is not enabled throughout the broad scope and that when the Wands factors are weighed it is concluded that undue experimentation would be required to practice the invention throughout the scope of the claims, and therefore the invention is not enabled.

Applicant submits that the guidance provided in the specification teaches rational modification of hordothionin and ESA polypeptides which can be used to practice the scope of the claimed invention. These genes and modifications are detailed supra.

Applicant submits that "the test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue." *In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 219 (CCPA 1976).

Based upon the guidance in the specification and the case law, applicant believes that the claims are commensurate in scope with teachings of the specification.

Rejections under 35 U.S.C. 112, second paragraph

The Examiner has rejected claims 1-18, 20, and 21 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention

The Examiner states that at Claim 1, line 5, the language "or combinations thereof and optionally" is confusing and indefinite. It is not clear what is encompassed by the claim. Appropriate correction is required.

Applicant submits that claim 1 has been rewritten and is believed to overcome the Examiner's rejection by reciting "... amino acids are lysine and a sulfur containing amino acid."

The Examiner states that at Claim 2, line 2, the language "or... and optionally..." is indefinite. It is not clear what is encompassed by the claim. Appropriate correction is required.

Serial No.: 09/020,718
Group Art Unit:

Applicant submits that Claim 2 has been cancelled.

The Examiner states that Claim 4 is indefinite in its recitation of "the preselected amino acid is lysine and a sulfur containing amino acid." It is unclear how the amino acid can be lysine and also be a sulfur containing amino acid. Appropriate correction is required.

Applicant submits that Claim 4 has been cancelled.

The Examiner states that at Claim 8, the phrase "the protein is ... derivatives of each protein" is improper English, due to the lack of agreement between the singular verb and plural direct object. Appropriate correction is required.

Agreement between objects and verbs has been corrected by rewriting original claim 8 as new claim 25.

The Examiner states that at Claim 8, line 3, "derivatives" is indefinite. There are many different types of derivatives, and Applicant has not clearly defined the term. Hence, it is not known what is encompassed by the claim. Appropriate correction is required to clarify the metes and bounds of the claimed invention.

Applicant submits that the term "derivatives" is defined in the instant specification on page 8 as "The derivatives differ from the wild-type protein by one or more amino acid substitutions, insertions, deletions or the like. Typically, amino acid substitutions are conservative." Pages 11 and 16 describe how derivatives are made through "nucleotide substitution of the structural gene so as to result in a polypeptide having a different amino acid at the position in the polypeptide which corresponds to the codon with the nucleotide substitution."

This definition provides the metes and bounds of the claimed invention.

The Examiner states that at Claims 10-12, line 2, the phrase "about ... percent by weight to about 10 times greater" is indefinite. The lower and upper ends of the range are in different units and the exact unit of the upper end of the range is unclear. Appropriate correction is required so that the lower and upper ends of the range are both definite and in the same unit of measurement.

Applicant submits that "10x greater" is equivalent to 1000 percent more by weight

Serial No.: 09/020,716
Group Art Unit:

(10x100) as now claimed in new claims 27-29.

The Examiner states that at Claim 13, line 1, and Claim 20, line 1, "seed endosperm-preferred promoter" should be changed to --seed endosperm-specific promoter" for clarity.

Applicant submits that support for seed-preferred promoters is found on page 6, lines 12-14 in the instant application. A "seed endosperm-preferred promoter" is defined as a promoter which preferentially promotes expression of the structural gene in the endosperm of the seed. Seed-specific promoter does not provide for leakiness or overlapping patterns of expression.

The Examiner states that at Claim 18, the phrase "seed product" is indefinite. It is not known what is intended by the phrase and/or what is encompassed by the claim. Appropriate correction is required to clarify the claimed invention.

Applicant submits that "seed product" is a known term and would be understood by one of skill in the art as that containing elevated levels of lysine and a sulfur containing amino acid.

The Examiner states that at Claim 18, the term "obtainable" is indefinite because it is not clear whether or not it is obtained. The term should be changed to --obtained--.

Applicant submits that original claim 18, rewritten as new claim 32, incorporates the Examiner's suggested change.

The Examiner states that at Claim 20, there is a lack of agreement between "seed" (singular) at line 1, and "seeds" (plural) at line 6. Appropriate correction is required.

Applicant submits that new claim 34 incorporates the Examiner's suggested changes.

The Examiner states that at Claim 20, there is a lack of agreement between "cell" (singular) at line 2, and "cells" (plural) at line 5. Appropriate correction is required.

Applicant submits that new claim 34 incorporates the Examiner's suggested change.

Serial No.: 09/020,716
Group Art Unit:

The Examiner states that Claim 20, line 5, the second method step "recovering the transformed cells" is incongruous with the first method step of "transforming a host plant cell," i.e. it is unclear what the transformed cell is recovered from. It is recommended that the second method step be deleted.

Applicant submits that new claim 34 incorporates the Examiner's suggested change.

The Examiner states that Claim 20, line 6 is unclear to what "therefrom" refers.

Applicant submits that new claim 34 removes the term in question.

Rejection Under 35 U.S.C. §102

Claims 1-21 are rejected under 35 U.S.C. 102(e) as being anticipated by Falco et al. (U.S. Patent 5,773,691).

The Examiner states that the claimed invention is indefinite for the reasons discussed supra, in particular, "derivatives" at Claim 8 is indefinite and reads on essentially any protein.

As stated *supra*, applicant submits that the term "derivatives" is defined in the instant specification on page 8 as "The derivatives differ from the wild-type protein by one or more amino acid substitutions, insertions, deletions or the like. Typically, amino acid substitutions are conservative." Pages 11 and 16 describe how derivatives are made through "nucleotide substitution of the structural gene so as to result in a polypeptide having a different amino acid at the position in the polypeptide which corresponds to the codon with the nucleotide substitution."

The Examiner states that Falco discloses transformed plants, especially corn, comprising seed with enhanced lysine content, obtained by expression of chimeric genes encoding lysine insensitive enzymes or lysine rich proteins (Abstract; Col. 1, lines 18-30; Col. 6, line 22 - Col. 7, line 44; Col. 9, line 38 - Col. 10, line 37; Col. 30, line 15 - Col. 31, line 62; Examples 22, 23, 25).

New claims 22, 30, 33 and 34 distinguish over Falco by requiring elevated levels of at least two amino acids; lysine and a sulfur containing amino acid.

Serial No.: 09/020,716
Group Art Unit:

New claims 22, 30, 33 and 34 further distinguish over Falco by requiring elevated levels in the endosperm. In column 88 Falco states "Lysine catabolism is expected to be much greater in the endosperm than the embryo and this probably prevents the accumulation of increased levels of lysine in seeds expressing Corynebacterium DHDPS plus E.Coli AKIII-M4 from the glutelin 2 promoter."

Claim 25 further distinguishes over Falco by requiring barley alpha hordothionin or 2S albumin proteins or derivatives.

Claim 30 further distinguishes over Falco by requiring a structural gene. Falco teaches biosynthetic pathway gene chimeras to elevate preselected amino acid.

The Examiner states that in particular, Applicant teaches said transformed plants wherein the increases in lysine are 10-400% (Col. 6, lines 65-66), and Applicant teaches use of an endosperm-specific promoter, including the zein promoter (Col. 19, lines 40-55). Hence, all of the claim limitations have been previously disclosed by Falco.

New claims 22, 30, 33 and 34 distinguish over Falco by requiring elevated levels of at least two amino acids, lysine and a sulfur containing amino acid.

New claims 22, 30, 33 and 34 further distinguish over Falco by requiring elevated levels in the endosperm.

Claim 25 further distinguishes over Falco by requiring barley alpha hordothionin or 2S albumin proteins or derivatives.

Claim 30 further distinguishes over Falco by requiring a structural gene.

Claim 30 further distinguishes over Falco by requiring a seed endosperm-preferred promoter operably linked to a structural gene. DHDPS is dihydridopicolinic acid synthase, which catalyzes the condensation of aspartyl B-semialdehyde with pyruvate in amino acid biosynthesis.

Claim 14 further distinguishes over Falco by requiring a gamma zein or waxy promoter.

Claim 32 further distinguishes over Falco by claiming a seed product.

Claim 33 further distinguishes over Falco by requiring a seed with lysine and a sulfur containing amino acid.

Claim 34 further distinguishes over Falco by requiring a seed endosperm-preferred promoter and a structural gene.

Serial No.: 09/020,716
Group Art Unit:

Furthermore, Falco teaches a biosynthetic pathway gene, which is insensitive to feedback inhibition by lysine as a means of increasing lysine levels.

In summary, the Falco reference does not disclose elevated levels of at least two amino acids, lysine and a sulfur containing amino acid, or elevated levels in the endosperm and therefore fails to anticipate the claimed invention.

Rejection Under 35 U.S.C. §102

Claims 1, 2, 4-13, 18 and 19 are rejected under 35 U.S.C. 102(e) as being anticipated by Rao (U.S. Patent 5,885,802).

The Examiner states that the claimed invention is indefinite for the reasons discussed supra. In particular, the claim language "or... and optionally" is indefinite, and hence the claims read on a transformed cereal plant seed with an elevated level of methionine. Also, the claimed increases in amino acid content in Claims 10-12 are not clear.

New claims 22, 30, 33 and 34 have been rewritten and do not contain the above-mentioned phrase.

New claims 27-29, respectively, clearly define the increases in amino acid content as "increased at least about 10 percent by weight to about 1000 percent by weight", "increased at least about 15 percent by weight to about 1000 percent by weight" and "increased at least about 20 percent by weight to about 1000 percent by weight", respectively.

Rejection Under 35 U.S.C. §103

Claims 1-21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rao et al. in view of Schernthaner et al. (EMBO 7:1249-1255, 1988).

The Examiner states that the claims are indefinite for the reasons discussed supra. However, it appears that Applicant broadly claims a transformed cereal plant seed with an elevated level of at least one amino acid, and particularly wherein said amino acid is lysine. Applicant also claims vectors comprising an endosperm-specific promoter operably linked to a gene which encodes a protein with an elevated level of an amino acid, a plant cell transformed therewith, and a method of enhancing the nutritional value of plant seed therewith.

Serial No.: 09/020,716
Group Art Unit:

The Examiner states that Scherthaner teaches the zein promoter, and teaches that the promoter has endosperm-specific activity (Abstract; Fig. 4).

Applicant submits that increasing the levels of lysine and a sulfur containing amino acid is not suggested or taught. Also, linking the zein promoter to a non-marker or structural, non-zein gene is not suggested.

Furthermore, Scherthaner teaches away from using the zein promoter for high levels of expression by the low levels of gene expression when the zein promoter was used as the promoter for the CAT marker.

It is noted that in order to establish a background for finding obviousness under 35 U.S. C. § 103 that the determination of the scope and contents of the prior art cannot be performed by the mere gathering of elements from separate and distinct disclosures irrespective of the teachings of the disclosures. There must be a reason apparent at the time the invention was made to select the particular combination or the references or the use of such teachings as evidence of obviousness with entail prohibited hindsight. *In re Nomiya*, 184 U.S.P.Q. 607 (CCPA 1975). One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention. *In re Fine*, 5 USPQ 2d. 1596 (Fed. Cir. 1988).

The Examiner states that it would have been prima facie obvious to modify the invention of Rao to substitute lysine rather than methionine in to the encoded hordothionin protein, because they are both nonpolar hydrophobic amino acids and because Rao teaches that threonine, lysine and methionine are all essential amino acids required for animal nutrition which are missing from and need to be increased in crop plants (Col. 1, lines 40-42; Col. 2, lines 8-10).

The Rao patent broadly discussed hordothionin modified with one amino acid. The present invention provides hordothionin modified with lysine and a sulfur containing amino acid.

Furthermore, the present invention distinguishes over Rao by teaching endosperm expression.

The Examiner states that it also would have been obvious to modify the invention of Rao to substitute an endosperm-specific promoter as taught by Scherthaner for the

Serial No.: 08/020,716
Group Art Unit:

constitutive promoter, because the invention was clearly directed to modification of seed tissue, and especially endosperm.

It is submitted that in view of Falco teaching away from expression in the endosperm, it would not have been obvious to substitute an endosperm promoter.

Furthermore, as discussed *supra*, Schernthaner's results teach away from using the zein promoter due to "weak seed-specific activity" (Abstract). The use of an endosperm promoter to increase levels of lysine and a sulfur containing amino acid is not suggested by Schernthaner.

The Federal Circuit has stated that "Obvious to try" does not constitute obviousness. *In re Deuel*, 51 F.3d 1552, 34 U.S.P.Q. 2d 1210, pg 1559 (Fed. Cir. 1995). Applicants believe that the same holding applies to this present application. *In re: Eli Lilly & Co.*, 902 F.2d 943, 14 U.S.P.Q. 2d 1741 (Fed. Cir. 1990).

The Examiner states that Rao's constitutive promoter and the endosperm promoters are functional equivalents, and it would have been obvious to substitute one functional equivalent for another.

The Examiners statement is respectfully traversed. Constitutive promoters and the endosperm-preferred promoters are not functional equivalents.

Furthermore, in light of Falco's teaching of greater lysine catabolism in the endosperm (Col 26, line 27-33 and Column 88 lines 37-41), applicant disagrees that it would have been obvious to substitute one promoter for the other.

The Examiner states that one would have had a reasonable expectation of success in view of the success of Rao.

Applicant respectfully reminds the Examiner that Falco teaches away from endosperm expression: "Lysine catabolism is expected to be much greater in the corn endosperm than the embryo and this probably prevents the accumulation of increased levels of lysine in seeds..." (Column 88 lines 37-41). This teaching removes expectation of success.

Serial No.: 09/020,716
Group Art Unit:

CONCLUSION

In light of the foregoing remarks, withdrawal of the outstanding rejection and allowance of all of the remaining claims is respectfully requested.

Respectfully submitted,



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